

# Synthesis of AZTp<sub>s</sub>p<sub>CX<sub>2</sub></sub>pp<sub>s</sub>A and AZTp<sub>s</sub>p<sub>CX<sub>2</sub></sub>pp<sub>s</sub>AZT: Hydrolysis-Resistant Potential Inhibitors of the AZT Excision Reaction of HIV-1 RT

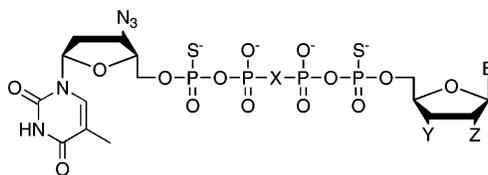
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## ABSTRACT



B = adenine, X = CH<sub>2</sub>, Y, Z = OH  
 B = adenine, X = CF<sub>2</sub>, Y, Z = OH  
 B = thymine, X = CH<sub>2</sub>, Y = N<sub>3</sub>, Z = H  
 B = thymine, X = CF<sub>2</sub>, Y = N<sub>3</sub>, Z = H

We report an efficient, one-flask route for synthesis of AZTp<sub>s</sub>p<sub>CX<sub>2</sub></sub>pp<sub>s</sub>A and AZTp<sub>s</sub>p<sub>CX<sub>2</sub></sub>pp<sub>s</sub>AZT, where X = H and X = F. This route makes use of the differential susceptibility to oxidation of H-phosphonate mono- and diesters, to allow a series of sequential reactions without requiring isolation of intermediates. These compounds are hydrolysis-resistant versions of the AZTppppA that results from excision of AZT by AZT-resistant HIV reverse transcriptase (RT). This family of compounds may therefore be useful in further study of the AZT excision reaction, as well as in drug design.

HIV-1 RT (human immunodeficiency virus type 1 reverse transcriptase) synthesizes DNA from the viral RNA strand and is a major target for anti-retroviral agents.<sup>1</sup> Nucleoside RT inhibitors (NRTIs), such as 3'-azido-3'-deoxythymidine (AZT), function as chain terminators and are used in clinical treatment of HIV infections.<sup>2</sup> Unfortunately, resistance to

such drugs commonly develops as the virus mutates. The principal mechanism for this resistance is enhanced removal of the chain-terminating nucleotide from the blocked DNA strand by an RT-catalyzed reaction that uses adenosine triphosphate (ATP) as a nucleophile.<sup>3</sup> This excision unblocks the DNA, permitting elongation to resume. AZT is particularly susceptible to this excision reaction because steric constraints from the azido group keep it in the nucleotide binding site of the catalytic center, where excision is favored.<sup>4</sup>

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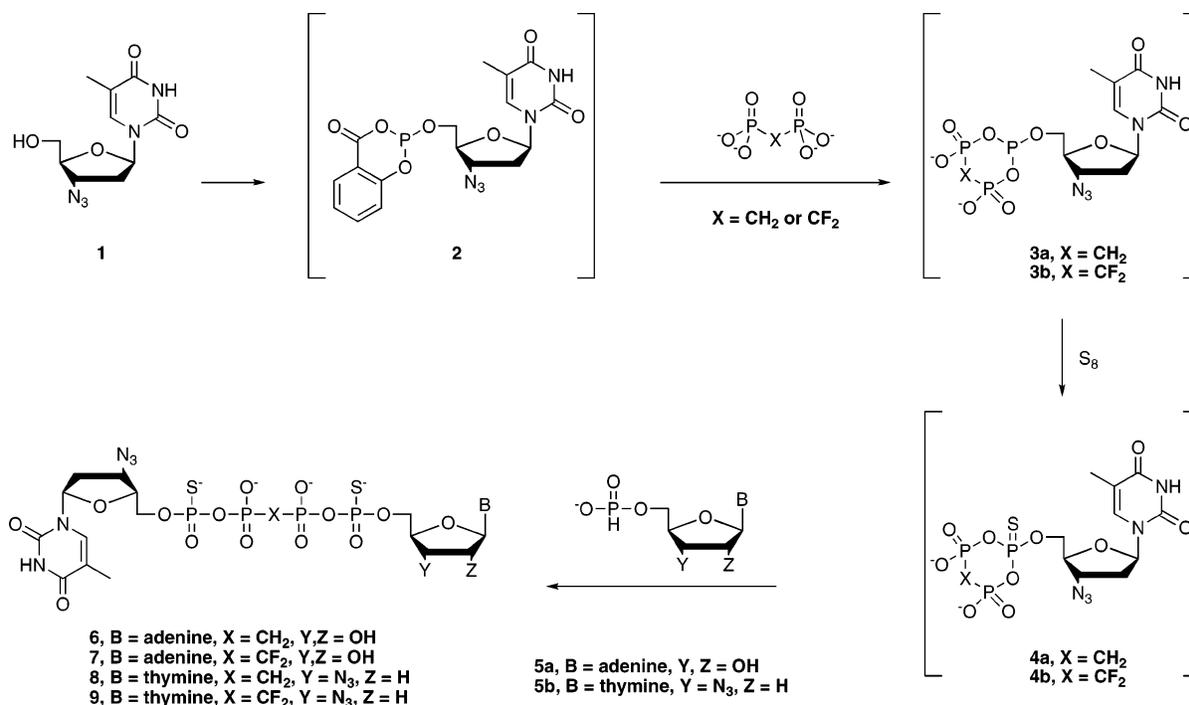
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Scheme 1



The dinucleoside tetraphosphate that results from excision of AZT is 5'-5'''-AZTp<sub>4</sub>A, which has recently been found to be an excellent chain-terminating substrate for resistant RT.<sup>5</sup> Hydrolysis-resistant versions of AZTp<sub>4</sub>A containing two outer thiophosphates and a central bisphosphonate, such as AZTp<sub>5</sub>PCX<sub>2</sub>pp<sub>5</sub>A, X = H (**6**) and X = F (**7**), and the related compounds with AZT at both ends, AZTp<sub>5</sub>PCX<sub>2</sub>pp<sub>5</sub>AZT, X = H (**8**) and X = F (**9**), may therefore serve as useful inhibitors of this excision reaction, thereby reducing AZT resistance.<sup>6</sup>

Thiophosphates as well as bisphosphonates are known to undergo reduced hydrolysis by cellular enzymes.<sup>7,8</sup> Therefore, these compounds should be resistant to such hydrolysis at all four phosphorus atoms and may lead to particularly promising candidates for long-lasting inhibitors of the AZT excision reaction. The adenosine in **6/7** may play a favorable role in tight binding to mutated forms of RT, while the presence of AZT at both ends of **8/9** may promote binding to wild type RT. Further, although both CH<sub>2</sub> and CF<sub>2</sub>

bisphosphonates are isosteric with phosphates, only CF<sub>2</sub> analogues are isopolar<sup>9</sup> and may enhance critical interactions at the binding site.

As part of our ongoing efforts to develop improved synthetic methods for dinucleoside polyphosphates and their analogues, we now report an efficient, one-flask synthesis of **6–9**. This route makes use of the ability of H-phosphonate diesters to undergo modification at the phosphorus atom, along with the differential susceptibility to oxidation of H-phosphonate mono- and diesters, to allow the series of sequential reactions shown in Scheme 1, without requiring isolation of intermediates. Because H-phosphonate monoesters are not easily oxidized, we can use **5a/b**, despite the presence of excess S<sub>8</sub> from the previous step, to condense with modified trimetaphosphates **4a/b**. It is only after the condensation has taken place that the excess S<sub>8</sub> in the reaction mixture then converts the product H-phosphonate diesters (not shown) to the dithiophosphates **6–9**.

The thiotrimetaphosphates **4a/b** were prepared by a route we recently reported for the synthesis of unmodified dinucleoside tetraphosphates.<sup>10</sup> Knorre some time ago developed a synthetic procedure for preparation of adenosine trimetaphosphate using a carbodiimide,<sup>11</sup> and this useful intermediate has been used by others to prepare a variety of nucleotide derivatives.<sup>12</sup> Eckstein introduced a particularly convenient method for preparing nucleoside trimetaphos-

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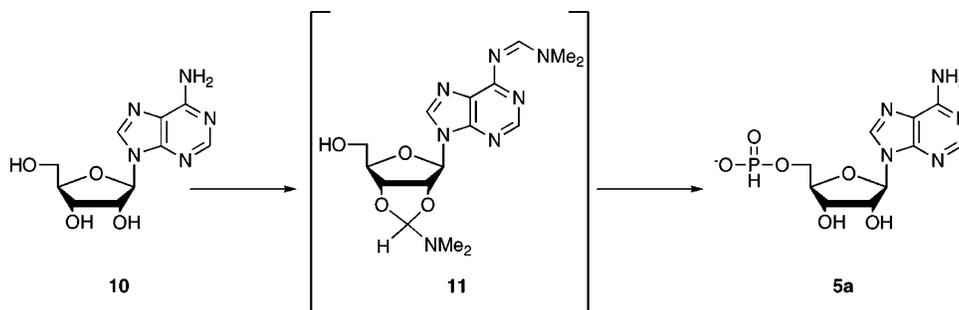
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## Scheme 2



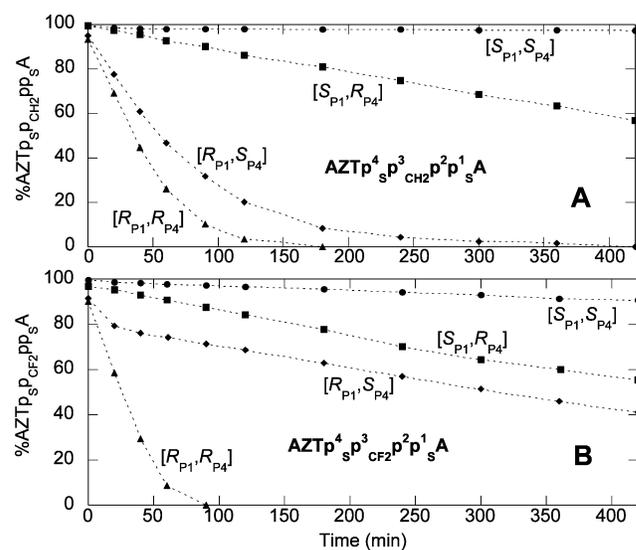
phates and  $\alpha$ -thionucleoside triphosphates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, followed by oxidation or sulfurization, respectively.<sup>13</sup> The procedure was further extended to prepare nucleoside triphosphates with various modifications<sup>14</sup> as well as with substituted  $\beta\gamma$ -methylenes along with  $\alpha$ -P-thioates.<sup>15</sup>

The first step in our procedure is reaction of a nucleoside, in this case AZT (**1**), with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one in DMF to give **2**. We then react **2** with a pyrophosphate analogue,<sup>16</sup> either methylene or difluoromethylene<sup>17</sup> diphosphonate, to form the modified trimetaphosphates, **3a** or **b**, respectively, followed by addition of excess  $S_8$  to give **4a/b**.

The 5'-H-phosphonates of either adenosine, **5a**, or AZT, **5b**, were made using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one.<sup>18</sup> To prepare adenosine H-phosphonate, **5a**, we first protect adenosine, **10**, using *N,N*-dimethylformamide dimethyl acetal to give **11** (Scheme 2).<sup>19</sup> This transient protection is conveniently reversed after the phosphitylation by overnight hydrolysis in dilute aqueous ammonia. The product is then converted to the tri-*n*-butylammonium form using cation exchange resin and dried so as to form a DMF solution that can be employed directly in the reaction with **4a/b**. AZT H-phosphonate, **5b**, does not require prior protection or conversion to the alkylammonium form. After the condensation, which is catalyzed by  $ZnCl_2$ , the second sulfurization is effected immediately by the excess  $S_8$  left from the first

sulfurization. We are able to separate all diastereomeric products, four each for unsymmetrical **6/7** and three each for symmetrical **8/9** (for which *RS* and *SR* are equivalent), with total isolated yields ranging from 57 to 82%. The related compounds  $Ap_{sp}CH_2pp_sA$ <sup>8</sup> and  $App_{CH_2}ppA$ <sup>20</sup> have previously been synthesized as hydrolysis-resistant versions of the signaling molecule  $Ap_4A$ , but in modest yields.

We have assigned the configurations of the diastereomers of **6–9** based on their relative susceptibility to hydrolysis by snake venom phosphodiesterase. Eckstein demonstrated that the *R* diastereomer of  $ppp_sA$  was cleaved significantly faster than the *S* diastereomer.<sup>21</sup> Further, he found that the *R* diastereomer had a longer retention time on reverse phase HPLC. Blackburn and McLennan used this selective enzymatic hydrolysis approach to assign configurations of the three diastereomers of  $Ap_{sp}CH_2pp_sA$ .<sup>8</sup> We have digested 0.94 mM samples of each diastereomer of **6–9** with 0.2–5 mg of snake venom phosphodiesterase and plotted the % undigested **6–9** remaining, determined by HPLC, as a function of time. The results for  $AZTp^4_{sp^3}CH_2p^2p^1_sA$  (**6a–d**) are shown in Figure 1A and demonstrate that the



**Figure 1.** Plots of % undigested tetraphosphates during enzymatic degradation for the diastereomers of  $AZTp^4_{sp^3}CH_2p^2p^1_sA$ , **6a–d** (panel A), and  $AZTp^4_{sp^3}CF_2p^2p^1_sA$ , **7a–d** (panel B).

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diastereomer with the longest retention time (**6d**) is cleaved faster than the others (26% left after 1 h), consistent with an  $R_{P1}, R_{P4}$  assignment. The diastereomer with the shortest retention time (**6a**) exhibits almost no cleavage, even after 7 h, consistent with an  $S_{P1}, S_{P4}$  assignment. Of the two remaining diastereomers with intermediate retention times, one (**6c**) is cleaved significantly faster than the other, with 46% left after 1 h. Because its cleavage produced almost entirely adenosine thiomonophosphate and adenosine (Supporting Information), we assign it  $R_{P1}, S_{P4}$ . The other intermediate diastereomer (**6b**) showed 93% left after 1 h and produced almost entirely AZT thiomonophosphate, consistent with an assignment of  $S_{P1}, R_{P4}$ .

Comparable degradation of  $AZTp^4s^3CF_2p^2p^1sA$  (**7**) required 2.5 times as much phosphodiesterase, and the results are shown in Figure 1B with corresponding assignments. Similar plots and assignments for **8/9** were done in the same fashion and are shown in the Supporting Information. In all four sets of diastereomers, the order of retention time on reverse phase chromatography correlates with the rate of enzyme degradation, with the compound showing the longest retention time degrading the fastest. The  $^{31}P$  NMR chemical shifts of all the diastereomers of **6–9** display extremely small differences, with no obvious correlation with configuration.

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For comparison, we have also synthesized the unmodified tetraphosphates,  $AZTp_4A$  (**12**) and  $AZTp_4AZT$  (**13**), and two analogues with central bisphosphonates but no thiophosphates,  $AZTpp_{CH_2}ppA$  (**14**) and  $AZTpp_{CF_2}ppA$  (**15**).<sup>22</sup> They show much faster degradation with snake venom phosphodiesterase than **6–9**, with only 50% left after about 1 min (Supporting Information).

This synthetic approach allows convenient one-flask synthesis of dinucleoside tetraphosphate analogues. The particular thiophosphate/bisphosphonate analogues described here are hydrolyzed by the well-known phosphodiesterase much more slowly than unmodified tetraphosphates, particularly their *SS* diastereomers. This family of compounds may therefore be useful in further study of the AZT excision reaction, as well as in drug design.

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**Supporting Information Available:** Synthetic methods, spectra (UV, MS,  $^1H$ ,  $^{13}C$ ,  $^{19}F$ , and  $^{31}P$  NMR), degradation methods, and plots of enzymatic degradation versus time. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(22)  $AZTp_4A$ ,  $AZTp_4AZT$ , and  $AZTppC_XppAZT$  ( $X = H, F$ ) were all made by our previously reported trimetaphosphate method using a nucleoside monophosphate.<sup>10</sup>  $AZTp_4AZT$  was also made by the H-phosphonate method reported here and gave comparable yields.